Chemical Constituents of *Coutaportla ghiesbregthiana*: Co-Crystallization of Two *ent*-Nor-Kaurene Diterpenes

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The known *nor*-diterpene (16R)-*ent*-17-hydroxy-19-*nor*-kaur-4-en-3-one and the new *nor*-diterpene (16R)-*ent*-19-*nor*-kaur-4-en-3-oxo-17-oic acid were obtained as a crystalline mixture from roots of *C. ghiesbregthiana*. Their structures were deduced by spectroscopic means and confirmed by a X-ray diffraction study of the crystalline mixture. Additionally, ursolic and betulinic acids, skimmin, and sucrose were isolated. This is the first chemical study of a member of the *Coutaportla* genus.

Key words: *Coutaportla ghiesbregthiana*, (16R)-*ent*-17-Hydroxy-19-*nor*-kaur-4-en-3-one, (16R)-*ent*-19-*nor*-Kaur-4-en-3-oxo-17-oic Acid, Triterpenes, Skimmin

Introduction

Until 1999 the genus *Coutaportla* (Rubiaceae) was constituted by three species *C. ghiesbregthiana*, *C. pailensis* and *C. guatemalensis* [1]. However, in 2003, *C. guatemalensis* was segregated into the new genus *Lorancea* as *L. guatemalensis* (Standl.) Borhidi [2]. Leaving *C. ghiesbregthiana*, and *C. pailensis* as the only two species in the *Coutaportla* genus. Both species are native to the new world, *C. ghiesbregthiana* is distributed from Northern Central America to Southern México [1] while *C. pailensis* grows in Coahuila, México [3]. Although several genera of Rubiaceae have been studied [4 – 6], to our knowledge no phytochemical study has been carried on *Coutaportla*.

As part of our ongoing systematic studies of Mexican plants [7], we want to report the study of *C. ghiesbregthiana*.

A chromatographic separation of the EtOAc roots extract of *C. ghiesbregthiana* led to the isolation of a mixture constituted by a known diterpene (16R)-*ent*-17-hydroxy-19-*nor*-kaur-4-en-3-one (**1**) and the new diterpene (16R)-*ent*-19-*nor*-kaur-4-en-3-oxo-17-oic acid (**2**). The structure of **2** was deduced by spectroscopic means. In order to resolve the mixture, a crystallization process was undertook, however it resulted in a co-crystal formed by **1** and **2**, which was analyzed by a X-ray diffraction study. We report in this paper the structural study of these diterpenes. In addition, ursolic (**3**) and betulinic (**4**) acids, skimmin (**5**), and sucrose (**6**) were also isolated.

Results and Discussion

The leaves, stem bark and roots of *C. ghiesbregthiana* were studied separately, thus the hexane, EtOAc and methanol extracts of each limb were obtained. In the Experimental Section, the isolation of **1 – 6** from different extracts is described. The identities of **1**, **3 – 6** were achieved by comparison of their physical and spectroscopic data with those previously reported [8 – 12].

In the IR spectrum of **1**/**2** mixture, ν OH absorption that starts at *ca.* 3500 cm^{-1} and extends to *ca.* 2300 cm^{-1}, was observed. However, there are several sub-maximal signals on this absorption, then the two bands at 3437 and 3391 cm^{-1} were assigned to the vibrations of the OH group of an alcohol moiety, while in the 3100 – 2300 cm^{-1} region characteristic bands due to an acid moiety arise from overtones or combination modes of internal vibrations, which overlap the νOH broad band and Fermi resonance between the ν OH fundamental of hydrogen bonds and overtone of its deformation modes. In the carbonyl region, the very

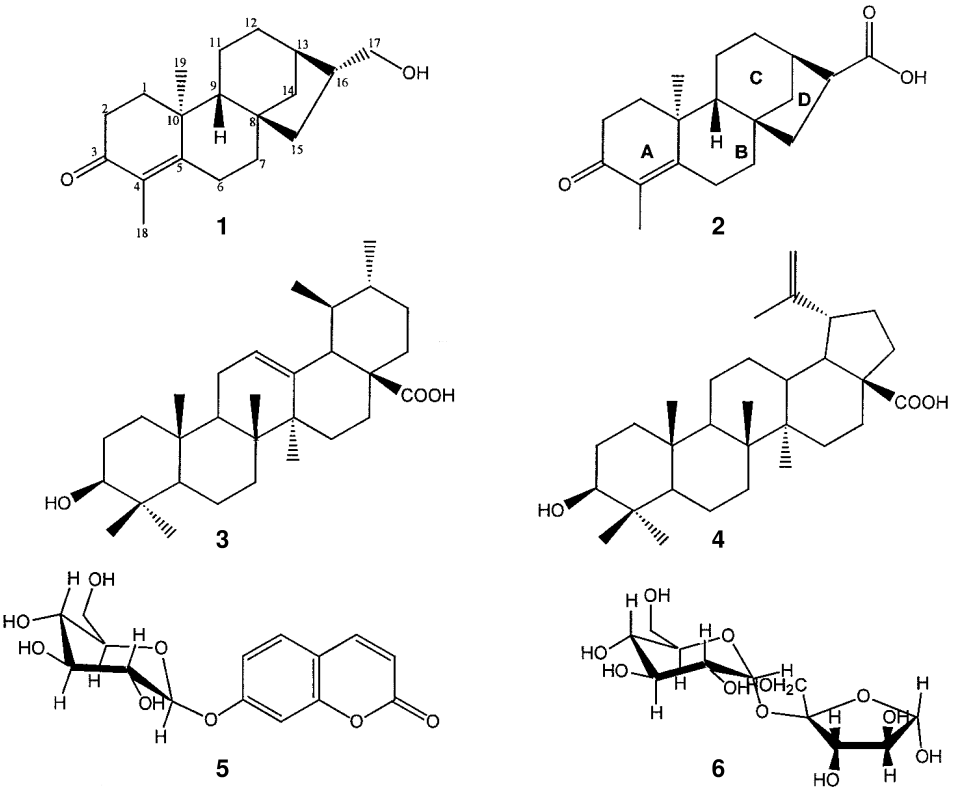

Table 1. ^{13}C NMR and DEPT spectral data for compound **2** (75 MHz, $CDCl_3$, δ-values).

Position	Chemical shift	DEPT	Position	Chemical shift	DEPT
C-1	37.16	CH2	C-2	33.30	CH2
C-3	198.84	C	C-4	128.34	C
C-5	163.69	C	C-6	26.97	CH2
C-7	38.22	CH2	C-8	44.81	C
C-9	53.85	CH	C-10	40.57	C
C-11	18.92	CH2	C-12	30.74	CH2
C-13	40.77	CH	C-14	36.76	CH2
C-15	43.45	CH2	C-16	45.08	CH
C-17	180.50	C	C-18	11.04	CH3
C-19	20.06	CH3			

strong bands at 1727 and 1642 cm^{-1} (accompanied with a shoulder at 1627 cm^{-1}) were assigned to the stretching vibration of the free C=O of an acid and a C=O moiety conjugated to a C=C (1594 cm^{-1}) group. Finally the *v* C-O vibrations for alcohol and acid moieties were assigned to the medium intensity bands observed at 1051 and 1190 cm^{-1}.

Although the **1**/**2** mixture always showed a spot in TLC, even when it was developed with different solvent mixtures the presence of **1** and **2** in the mixture was evidenced in the ^{1}H and ^{13}C NMR spectral data. However, since the quantities of **1** and **2** were almost the same, no reliable data could be obtained from the ^{1}H NMR spectrum except those assigned to H-16 at 3.17 ppm for **1**. However, the signals for **1** and **2** could be assigned in the ^{13}C NMR spectra.

The ^{13}C NMR spectrum of **1**, showed the following signals: a carbonyl at $\delta_C = 198.99$, two olefinic carbons at $\delta_C = 128.15$ and $\delta_C = 164.23$, two methyl carbons at $\delta_C = 20.12$ and $\delta_C = 11.04$, eight methylene carbons ($\delta_C = 44.03$, 38.84, 36.79, 36.44, 33.30, 31.06, 26.97 and 19.16), a hydroxymethylene carbon at $\delta_C = 67.33$ and two methine carbons at $\delta_C = 54.14$ and $\delta_C = 43.34$. The structure **1** was deduced by the comparison of its ^{13}C NMR spectrum with those reported in the literature [12] as well as spectroscopic data of (16S)-*ent*-16,17-dihydroxy-19-*nor*-kaur-4-en-3-one [6].

For compound **2**, 19 signals in ^{13}C NMR spectrum were observed (Table 1), two carbonyl at $\delta_C = 198.84$ (ketone) and $\delta_C = 180.50$ (acid), two olefinic carbons at $\delta_C = 128.34$ and $\delta_C = 163.69$, two methyl carbons at $\delta_C = 20.06$ and $\delta_C = 11.04$, eight methylene carbons ($\delta_C = 43.45$, 38.22, 37.16, 36.76, 33.30, 30.74, 26.97

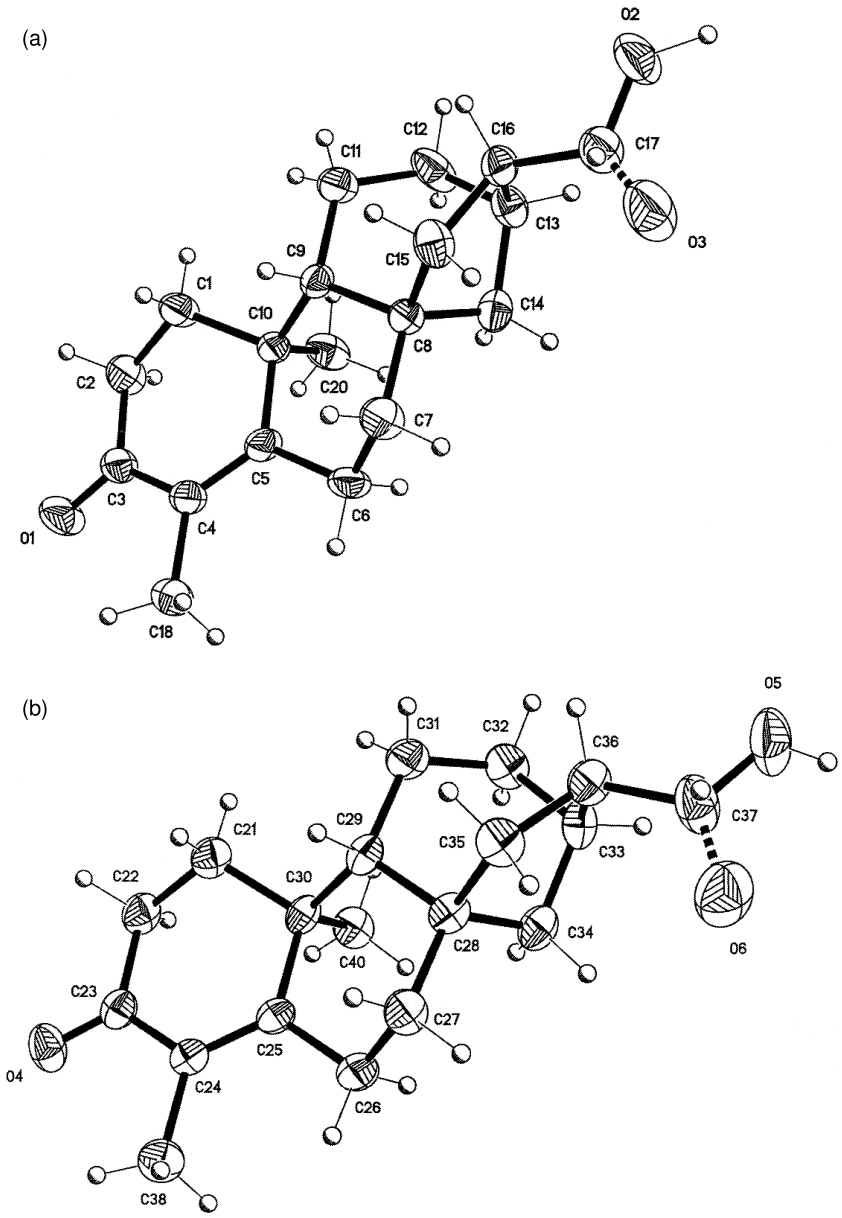

Fig. 1. Perspective view of the two independent molecules co-crystal of **1** and **2**. Thermal ellipsoids are drawn at 30% probability levels.

and 18.92) and three methine carbons at $\delta_C = 53.85$, $\delta_C = 45.08$ and $\delta_C = 40.77$ (Table 1). In the high-resolution mass spectrum, the ion molecular peaks [M+] at *m/z* at 302.4059 ($C_{19}H_{26}O_3$) corresponding to **2** was observed.

Since separation of both **1** and **2** by standard chromatographic methods failed, in order to purify at least one component of the mixture, a crystallization process was carried out. Then adequate crystals suitable for X-ray analysis were obtained. Though one-compound crystal was expected, the structure of the crystal showed the presence of both **1** and **2**.

The structure of a mixed crystal of **1** and **2** in an approximate 1 : 1 ratio was determined. The unit cell contains two independent crystallographic molecules, here and after, molecule A (C-1 to C-19, O-1 to O-3) and molecule B (C-20 to C-38, O-4 to O-6). Both molecules A and B appeared as superimposed structures of statistically disordered pairs of **1** and **2** (Fig. 1). The relative contributions [Molecule A: 0.652(5) alco-

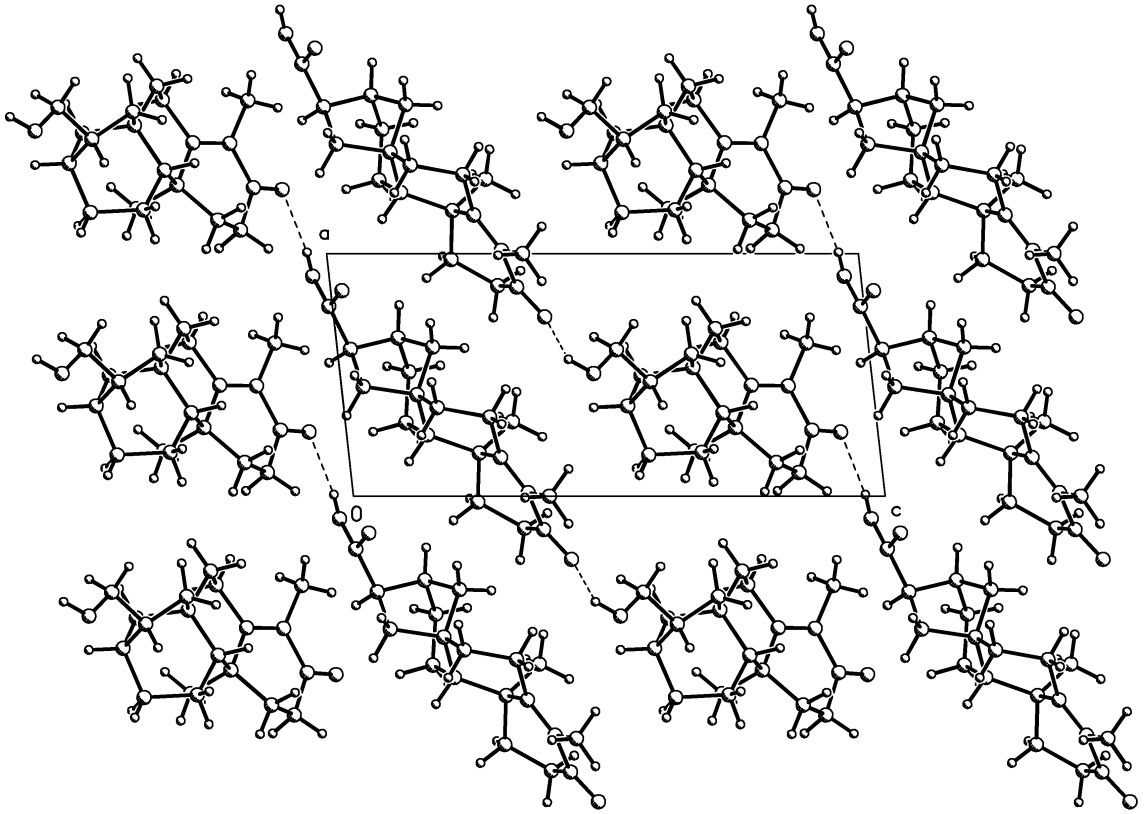

Fig. 2. Crystal packing of **1/2** projected onto the *ac* plane. Thick and thin lines indicate A and B molecules, the dashed lines in indicate the hydrogen bonds.

hol **1**: 0.348(5) acid **2**; Molecule B: 0.348 (5) alcohol **1**: 0.652 (5) acid **2**] were determined by refinement of the occupancy of the oxygen atoms O-3 and O-5. None withstanding the observed statistically disorder, the average U_{eq} calculated over all non-hydrogen atoms is 0.0559 Å^2 (minimum: 0.0421 and maximum: 0.0785 Å^2) revealing that the carbon framework of **1** and **2** are essentially identical.

All bond lengths in the structures are as expected. Selected values of the molecular geometry are reported in Table 4. Differences between the corresponding bond lengths of A and B molecules are in average within the experimental errors (three times the e.s.d.'s).

Ring conformations are very similar in the two independent molecules. The $\Delta^{4,5}$ cyclohexene ring A, the cyclohexane ring B and the five-member ring D are close to the ideal sofa, chair and envelope conformations, respectively. The atomic displacements for cyclohexene rings are C1 = −0.323 Å in Molecule A and C20 = 0.315 Å in Molecule B while in the five-member rings are C14(33) = −0.300 Å, with respect to the best plane through the remaining ring atoms.

Cyclohexane ring C takes conformations intermediate between chair and half-chair forms. Deviations from the ideal C_2 (chair) and C_S (half-chair) symmetries can be evaluated by the asymmetry parameters [13]: ΔC_2 (C9-C11) = 8.4°, ΔC_S(C11) = 5.9°, and ΔC_2(C28-C30) = 11.1°, ΔC_S(C30) = 3.1°. For all these rings the puckering parameters [14] are shown in Table 5.

Due to the insignificant degree of anomalous scattering, the chirality of the structures were assigned from the known configuration at C-16, the relative stereochemistry of the structures referred to this center and shown in Fig. 1 is 8S, 9S, 10S, 13R and 16R.

The crystal packing of **1**/**2** is shown in Fig. 2. Molecules A and B are interconnected in a head to tail fashion by hydrogen bonds between hydroxyl (from the carbinol or the acid moieties) groups and the ketone carbonyl O-1 and O-4 oxygen atoms (Table 6), forming infinite rows along the [2 2 −1] direction.

Table 2. Summary of crystal data and structure refinement for mixed crystal.

Empirical formula	$C_{38}H_{54}O_5$ [($C_{19}H_{28}O_2$) + ($C_{19}H_{26}O_3$)]
Formula weight	590.81
Crystal system	triclinic
Space group	*P*1
Unit cell dimensions	$a = 7.639(1)$ Å $\alpha = 92.383(2)^\circ$ $b = 7.660(1)$ Å $\beta = 94.780(2)^\circ$ $c = 15.172(1)$ Å $\gamma = 114.262(2)^\circ$
Volume [Å^3]	803.7(2)
Z	1
Density (calculated) [mg/m^3]	1.225
Absorption coefficient [mm^{-1}]	0.079
F(000)	324
θ Range for data collection [°]	2.70 to 25.00
Index ranges	$-9 \le h \le 9$, $-9 \le k \le 9$, $-18 \le l \le 17$
Completeness to $\theta = 25.00^\circ$ [%]	100.0
Data / restraints / parameters	5297 / 3 / 408
Goodness-of-fit on F^2	0.991
Final *R* indices [$I > 2\sigma(I)$]	$R1 = 0.0474$, $wR2 = 0.0557$
R Indices (all data)	$R1 = 0.0843$, $wR2 = 0.0612$
Absolute structure parameter	0.4(12)
Largest diff. peak and hole [e.Å^{-3}]	0.151 and −0.149

Conclusion

C. ghiesbregthiana has been shown to contain the known diterpene **1**, the new diterpene **2**, ursolic (**3**) and betulinic (**4**) acids, skimmin (**5**) and sucrose (**6**). To our knowledge, this is the first phytochemical study of a member of the *Coutaportla* genus.

The structures of **1** and **2** have been determined by a X-ray diffraction analysis from a binary co-crystal. This result confirms our spectroscopic findings and also permits us to establish that the co-crystal is a mixed crystal (solid solution) with a disordered molecular arrangement. In the mixed crystal, the random occupation of molecules of the two different compounds covers all possible combinations of the intermolecular relationship, which is similar to that of a solution. Thus, we may deduce that, in solution, a pre-organized **1**/**2** adduct exists which is more soluble than the single compounds **1** and **2**, favoring the co-crystallization over crystallization of the individuals.

The presence of **1** and **2** in *C. ghiesbregthiana* and (16S)-*ent*-16,17-dihydroxy-19-*nor*-kaur-4-en-3-one in *Exostema acuminatum* [6] support the proposal that *Coutaportla* and *Exostema* are very close genera [15].

Experimental Section

General

The melting points (uncorrected) were determined on a Fisher-Johns apparatus. The Infrared spectrum was measured on a Bruker Tensor 37 spectrometer. The solid-state spectrum was recorded in Nujol suspension on NaCl plates. ^{1}H NMR, ^{13}C NMR and ^{1}H-^{1}H COSY (including DEPT) spectra were measured on a Varian Gemini XR-300 instrument operating at 300 and 75 MHz respectively. The mixture was analyzed in $CDCl_3$with tetramethylsilane (TMS), as internal standard.

Table 3. Fractional atom coordinates ($\times 10^4$) and equivalent thermal parameters ($\times 10^3$ Å^2) of molecules A and B in the mixed crystal.

Atom	*x*	*y*	*z*	U_{eq}	Atom	*x*	*y*	*z*	U_{eq}
O1	2650(4)	3548(4)	9297(2)	81(1)	O4	−2596(4)	8153(3)	3968(2)	67(1)
O2	5049(4)	15086(4)	4770(2)	91(1)	O5	9100(4)	10691(4)	−308(2)	95(1)
O3	7639(7)	14537(7)	5161(3)	129(2)	O6	8500(12)	13248(11)	214(6)	100(4)
C1	1389(5)	6849(5)	8023(2)	61(1)	C20	−239(4)	6624(4)	2323(2)	56(1)
C2	985(5)	4959(5)	8427(2)	68(1)	C21	−1316(5)	6400(4)	3144(2)	57(1)
C3	2732(5)	4721(5)	8761(2)	52(1)	C22	−1366(5)	8211(5)	3500(2)	47(1)
C4	4551(5)	5880(4)	8403(2)	47(1)	C23	141(5)	10046(4)	3290(2)	43(1)
C5	4584(4)	6933(4)	7708(2)	48(1)	C24	1597(5)	10092(4)	2845(2)	44(1)
C6	6349(5)	7821(5)	7230(2)	65(1)	C25	3267(4)	11978(4)	2724(2)	60(1)
C7	6909(4)	9956(5)	7157(2)	66(1)	C26	3663(5)	12160(4)	1757(2)	57(1)
C8	5305(4)	10347(4)	6651(2)	42(1)	C27	4193(5)	10592(4)	1405(2)	44(1)
C9	3441(4)	9368(4)	7119(2)	43(1)	C28	2526(4)	8612(4)	1516(2)	44(1)
C10	2832(4)	7237(4)	7325(2)	42(1)	C29	1783(4)	8312(4)	2452(2)	42(1)
C11	1815(4)	9785(5)	6647(2)	66(1)	C30	3036(5)	6989(4)	1138(2)	57(1)
C12	1709(5)	9754(5)	5643(2)	77(1)	C31	5141(5)	7309(4)	1304(2)	60(1)
C13	3731(5)	10820(5)	5347(2)	54(1)	C32	6540(5)	9377(5)	1165(2)	54(1)
C14	4979(5)	9809(4)	5660(2)	54(1)	C33	6159(5)	10750(4)	1798(2)	55(1)
C15	5860(5)	12529(4)	6650(2)	58(1)	C34	4528(5)	10753(4)	414(2)	57(1)
C16	4724(5)	12816(4)	5824(2)	53(1)	C35	6057(5)	9958(4)	252(2)	56(1)
C17	6005(6)	14273(5)	5248(3)	66(1)	C36	7847(6)	11442(6)	−64(3)	79(1)
C18	6325(4)	5661(5)	8833(2)	62(1)	C37	3(5)	11822(4)	3684(2)	61(1)
C19	1872(4)	5804(4)	6517(2)	63(1)	C38	3082(4)	7788(4)	3137(2)	61(1)

Table 4. Selected bond lengths [Å] and angles [°] with e. s. d. in parentheses.

O1–C3	1.223(3)	O4–C22	1.212(4)
O2–C17	1.330(4)	O5–C36	1.371(4)
O3–C17	1.199(5)	O6–C36	1.300(8)
C4–C5	1.350(4)	C23–C24	1.338(4)
C5–C4–C3	121.4(3)	C24–C23–C22	121.1(3)
C5–C4–C18	123.8(3)	C24–C23–C37	123.6(3)
C3–C4–C18	114.7(3)	C22–C23–C37	115.0(3)
C4–C5–C6	121.8(3)	C23–C24–C25	121.0(3)
C4–C5–C10	123.1(3)	C23–C24–C29	124.6(3)
O3–C17–O2	123.1(4)	O6–C36–O5	119.9(5)
O3–C17–C16	124.3(5)	O6–C36–C35	121.4(5)
O2–C17–C16	112.2(4)	O5–C36–C35	112.6(3)

Table 5. Cremer and Pople parameters of molecules A and B in the mixed crystal.

	q_2 (Å)	q_3 (Å)	Q_T (Å)	θ (°)	φ (°)
Ring A (molecule A)	0.4031	−0.2201	0.4593	119	179
Ring A (molecule B)	0.3777	−0.2360	0.4454	122	180
Ring B (molecule A)	0.0936	−0.5215	0.5298	170	287
Ring B (molecule B)	0.1179	−0.5135	0.5268	167	293
Ring C (molecule A)	0.2305	0.5849	0.6287	22	286
Ring C (molecule B)	0.2303	0.05738	0.6184	22	292
Ring D (molecule A)	0.4767	—	0.4767	—	222
Ring D (molecule B)	0.4754	—	0.4754	—	216

Table 6. Hydrogen bonds between Molecules A and B in the mixed crystal.

D-H…A	d(D-H), Å	d(H…A), Å	d(D…A), Å	⟨(DHA), °
O2-H2…O4^{I}	1.05(4)	1.75(4)	2.714(3)	151(3)
O5-H5…O1^{II}	0.85(4)	1.99(4)	2.829(3)	171(4)

Symmetry transformations used to generate equivalent atoms:^{I} $x+1$, $y+1$, z; ^{II} $x+1$, $y+1$, $z-1$.

^{13}C NMR multiplicity was determined using DEPT experiments. The EIMS and HRMS were recorded on a JEOL JMS-SX102A Instrument.

Plant material

C. ghiesbregthiana was collected in Tehuacán, Puebla, Mexico and identified by Dr. N. Diego (Facultad de Ciencias, UNAM). A voucher specimen (FCME 76921) is on deposit at the herbarium of the Facultad de Ciencias, UNAM, Mexico.

The plant material was dried and separated into leaves (946 g), stem bark (702 g) and roots (609 g). Each part was extracted successively with hexane, EtOAc and methanol at room temperature. The extracts were evaporated under reduced pressure to dryness.

All the extracts were chromatographed using an open column packed with Si-gel (G-Altech, 0 – 0.5 mm, ASTM) in a 1 : 30 proportion to the extract and eluted with solvent mixtures of increasing polarity starting with hexane and ending with methanol.

Leaves. When the EtOAc extract (54.5 g) was treated with methanol, a solid precipitated which was filtered and exhaustively washed with methanol affording impure ursolic acid (**3**). Purification of **3** (17 g, m. p. 279 – 281 °C) [8] was achieved by recrystallization from hexane-EtOAc. The residual extract was chromatographed, a total of 128 fractions of 100 ml each were collected. Fractions with identical TLC were combined. Ursolic acid (**3**, 0.346 g) was isolated from the combined fractions 40 – 78 (hexane-EtOAc, 1 : 1).

When the methanol extract (79 g) was tried to re-dissolve with methanol, a solid precipitated and was filtered and exhaustively washed with methanol affording ursolic acid (**3**, 5.6 g). The resulting solution was slowly evaporated to dryness at room temperature. When it was treated with methanol, impure skimmin (**5**) precipitated. It was isolated by filtration and washed with methanol affording **5** pure (0.72 g, m. p. 207 – 209 °C) [9]. Its mother liquors were chromatographed collecting a total of 80 fractions of 100 ml each. Fractions with identical TLC were combined. From the fractions 54 – 63 eluted with hexane-EtOAc (2 : 8) skimmin (**5**, 2 g) was isolated while sucrose (**6**) [10] was isolated from the fraction 64, eluted EtOAc-methanol (1 : 1).

Stem bark. The chromatography of the hexane extract of the stem barks (4.23 g) provide a total of 20 fractions. Betulinic acid (**4**, 0.015 g) [11] was isolated from the fractions eluted with hexane-EtOAc (8 : 2). From the methanol extract (68 g), ursolic acid was isolated (0.094 g) using similar chromatographic procedures.

Roots. The chromatography of the roots EtOAc extract (6 g) affording a total de 80 fractions. The **1**/**2** mixture (0.038 g, m. p. 150 – 155 °C) was isolated, as amorphous precipitate, from combined fractions eluted with mixtures of hexane/EtOAc (8 : 2). Adequate crystals with appearance as plates suitable for X-ray analysis were obtained by crystallization. The structure of these plates showed the presence of both compounds, *i.e.* the alcohol (**1**) and the acid compound (**2**). The methanol extract (44.52 g) was chromatographed yielding a total of 50 fractions. From the reunited fractions 7 – 25 eluted with EtOAc Skimmin (**2**, 7.9 g) was isolated [9].

The structures of the known compounds were established by comparing spectral and physical data obtained with those reported in the literature [8-12].

Mixture of diterpenes **1** *and* **2**

M. p. 150 – 155 °C. MS (EI, 70 eV): *m/z* (%): 302(30), 288(95), 273(34), 176(12), 138(53), 136(100), 121(22) and 91(33). IR (Nujol) ν(cm^{-1}) 3437, 3391, 1727 (C=O), 1642 (C=O), 1051, 1190. ^{13}C NMR for **2** see Table 1.

X-ray diffraction analysis

A colorless lamina-like crystal of the co-crystal of **1** and **2**, of approximate dimensions 0.28 mm × 0.12 mm × 0.07 mm,

was used for X-ray crystallographic analysis. The X-ray intensity data were measured at 298 K on a Bruker SMART APEX CCD-based X-ray diffractometer system equipped with a Mo-target X-ray tube ($\lambda = 0.71073$ Å). The detector was placed at a distance of 4.837 cm from the crystal.

A total of 3000 frames were collected with a scan width of 0.3° in ω and an exposure time of 30 sec/frame. The frames were integrated with the Bruker SAINT software package [15] using a narrow-frame integration algorithm. The integration of the data using a triclinic unit cell yielded a total of 9529 reflections to a maximum 2θ angle of 50.00° (0.84 Å resolution), of which 5297 were independent (redundancy 3.38, $R_{int} = 3.74\%$, $R_{sig} = 9.04\%$) and 3148 were greater than $4\sigma(F)$. The final cell constants are based upon the refinement of the XYZ-centroids of 3060 reflections above $20\sigma(I)$. Analysis of the data showed negligible decay during data collection, the intensities were corrected for Lorentz and polarization factors and an empirical absorption correction was applied *via* ($T_{min} = 0.9843$, $T_{max} = 0.9947$).

The structure was solved and refined on F^2 values by full-matrix least squares with anisotropic thermal parameters for non-hydrogen atoms, using the Bruker SHELXTL (Version 6.10) Software Package [16], in the space group $P1$, with $Z = 1$ for the formula unit $C_{38}H_{54}O_5[(C_{19}H_{28}O_2) + (C_{19}H_{26}O_3)]$. Occupancies for the C=O atoms O-3 and O-5 were permitted to refine freely at initial stages, in the final cycles of refinement they were paired to sum unity. Positions of hydrogen atoms attached to O-atoms were found from the difference Fourier map and their coordinated refined. The rest of H atoms in this structure were introduced at calculated positions as riding atoms, with C-H = 0.98 (CH), 0.97 (CH_2) or 0.98 Å (CH_3) and U_{iso}(H) = 1.2 U_{eq}(C). The crystal data and details concerning data collection, structure refinement, atomic coordinates and equivalent isotropic displacement parameters are given in Tables 2 and 3.

Crystallographic data for the structure(s) have been deposited with the Cambridge Crystallographic Data Center, CCDC-232743. Copies of the data can be obtained free of charge on application to The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: int.code+(1223)336-033; e-mail for inquiry: fileserv@ccdc.cam.ac.uk).

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[1] D. H. Lorence, in V. C. Hollowell, M. R. Crosby (eds): Monographs in systematic botany from Missouri Botanical Garden, Vol. 73, Missouri Botanical Garden Press, St Louis Missouri (1999).
[2] A. Borhidi, Act. Bot. Hung. **45**, 13 (2003).
[3] Q. J. A. Villarreal, Sida **12**, 223 (1987).
[4] H. Inouye, Y. Takeda, H. Nishimura, A. Kanomi, T. Okuda, C. Puff, Phytochem. **27**, 2591 (1988).
[5] J. Rockenbach, A. Nahrstedt, Planta Med. **56**, 591 (1990).
[6] A. Ito, H. Chai, Y. G. Shin, R. García, M. Mejía, Q. Gao, C. R. Fairchild, K. E. Lane, A. T. Menéndez, N. R. Farnsworth, G. A. Cordell, J. M. Pezzuto, A. D. Kinghorn, Tetrahedron **56**, 6401 (2000).
[7] M. Martínez-Vázquez, A. N. García-Argáez, in S. G. Pandalai (ed): Recent Res. Devel. Phytochem. Vol. 5, p. 59-85 ED, Research Signpost, India (2001).
[8] P. J. Houghton, L. L. Ming, Phytochem. **25**, 1939 (1986).
[9] N. J. Cussans, T. N. Huckerby, Tetrahedron **31**, 2719 (1975).
[10] C. J. Pouchert, J. Behnke, The Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra, **Vol. 1**, Aldrich Chem. Co., USA (1993).
[11] R. Argumedo D., H. Parra-Delgado, T. Ramírez A., A. Nieto C., M. Martínez-Vázquez, Rev. Soc. Quim. Mex. **47**, 167 (2003).
[12] A. A. Ahmed, T. A. Hussein, A. A. Mahmoud, M. A. Farag, P. W. Paré, M. Wojcinska, J. Karchesy, T. J. Mabry, Phytochem. **65**, 2539 (2004).
[13] W. L. Duax, C. M. Weeks, D. C. Rohrer, in N. L. Allinger, E. L. Eliel (eds): Top. Stereochem., **Vol. 9**, p. 271 – 374, John Wiley & Sons, New York (1976).
[14] D. Cremer, J. A. Pople, J. Am. Chem. Soc. **97**, 1354 (1975).
[15] J. H. E. Rova, P. G. Delprete, L. Anderson, V. A. Albert, Am. J. Bot. **89**, 145 (2002).
[16] Bruker, SMART (Version 5.625), SAINT-Plus (Version 6.23C) and SHELXTL (Version 6.10). Bruker AXS Inc., Madison, Wisconsin (1999).